



e-Network Forum

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What are best practices for building a frozen serum repository of blood donor samples for possible future infectious agent testing?

A member of a Swiss working group that concerns itself with questions related to transfusion-transmitted diseases reports that it is a requirement in Switzerland for an aliquot of **0.9 ml** of serum or plasma from each blood donation to be stored frozen for 5 years. The above sample retention requirement applies to all allogenic donations, including apheresis (platelets, plasma). There is an ongoing discussion about the requirements for pipetting the retention sample. The Swiss colleague **believes that it should be a requirement to pipette each sample with a new tip**, to exclude carry-over, since it is not known today with which assays or methods one might want to test these samples in the future, especially if NAT testing is involved or a test for Prions to detect vCJD becomes available. Some people oppose this requirement, because their automatic pipetting system does not have the capability to change pipette tips; rather, carry-over is prevented by employing wash cycles. The Swiss colleague says that this can be validated to be adequate for today's assays, but wonders if it will be adequate for future assays? The Swiss colleague wonders what are the requirements in other countries as well as what are the views of other colleagues?

Before sending the above query to the full e-network, it was **previewed by a recognized expert US scientist** who is intimately involved with blood donor screening for infectious diseases. She strongly recommends a new pipette tip for each donor sample. She justifies this recommendation by pointing out that qualification of wash steps, even for many of today's assays, doesn't work. She offered two examples:

- **HBsAg** occurs in samples in high concentrations and can be demonstrated to be carried over to the next one to three wells from a strongly positive sample following the "qualified" washes on at least one kind of automated pipettor. Accumulation of HBsAg can be seen on these tips via EM (after washing).
- Her group has repeatedly demonstrated **anti-CMV** carry-over on test instruments, again after the qualified wash steps.

The above two analytes are not unique, and certainly it could be argued that for NAT, the requirements should be even stricter. She would imagine similar concerns would apply for vCJD.

Finally, she commented that the 0.9 mL sample volume being used for building the repository may be too small. In her experience, for NAT, the test requires 0.5 mL for a multiplex test with 0.6 mL dead volume required for pipetting. Certainly, all test volume requirements will vary, but the US scientist would suggest saving at least 1.5 to 2.0 mL if she were building a repository. The costs for creating a repository are high, and in her opinion, to sacrifice this with too small a sample volume or with less than optimal pipetting processes does not make good scientific sense.

The following responses have been received.

ADDENDA Dec. 2, 2002

1. **A second US expert** on blood donor disease screening agrees with the first expert's recommendation for pipette tip-changing. In this expert's opinion, the dynamic range for some analytes is enormous and well beyond the capabilities of most, if not all, wash systems. Consider, for example, peak titers of the B19 erythrovirus (parvovirus) which have been quoted at 10^{12} per mL. Even for serology, HBsAg may be detected at at least a million-fold dilution. The first expert's example of CMV is very telling - any samples that have been handled by at least one manufacturer's instrument (which is not designed to prevent cross-contamination with high titer analytes) are, in the second expert's opinion, unsuitable for infectious disease testing.
2. **A senior scientist in San Francisco** is of the opinion that with the increasing sensitivity of new assays, any contamination of repository samples could result in a repository being worthless. A lot of time and money goes into maintaining a long-term repository. Therefore, it would be a waste if when the time arrives for its intended use, it is found to be worthless.

ADDENDA Dec. 3, 2002

3. **Another senior US researcher** with significant experience in donor screening reports that in his experience it is also important to ensure that samples are not damaged by multiple freeze-thaw cycles. To reduce this possibility, his group saves samples from any one donor in smaller aliquots and tries to use different volumes where possible. For instance, if they have 3 ml of serum from a donor for a repository they will likely save two 0.5 ml samples and then two 1 ml samples. Although this uses more freezer space, in his opinion, the benefit of having uncompromised reserve samples is worth the effort.

ADDENDA Dec. 8, 2002

4. **A hematologist working in Spain** as a quality manager of a donor center reports that in his country donor centers usually keep frozen repositories for every blood or plasma donation, and that the duration of samples in the repository is indefinite. Since there is no legal specification, **each Spanish center follows their own local policy/procedure**. Some centers use "new tip" devices, others use repeated washes. The volumes frozen, the recipient and the storage temperature are also very heterogeneous. The reporting hematologist's center has used both kind of devices; presently they change tips with every sample. They used to keep 400 microliters divided in two aliquots, using microtiter plates. Now they dispense 1 ml in a deep, Eppendorf-like plate and 300 microliters in a microtiter plate. They don't have the room or the time to keep larger samples. The hematologist feels that at least 2 ml should be kept, aliquoted in two or three samples and that a new tip should be used for every sample. This, is nevertheless not feasible thinking, given that one has to work with real budgets.

ADDENDA Dec. 9, 2002

5. **The scientist in San Francisco (#2 above)** agrees with the researcher who suggested that **multiple** aliquots be saved so that the amount of freeze/thawing can be reduced. However, since Gen-Probe/Chiron-based NAT testing requires 0.5 mL, he would make the size of the aliquots **0.6 mL** instead of 0.5 mL, in order to **ensure** recovery of at least 0.5 mL from the storage tube.

Please submit comments to the [e-Network Forum](#).

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Addenda: Dec. 2, 3, 8 & 9, 2002

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